BMP-2-Incorporated Biomimetic Coating-Functionalized Biomaterials for Bone Tissue Engineering

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Chapter 8

Summary

The repair of critical-sized bone defects remains a challenge in the fields of implantology, maxillofacial surgery and orthopedics. Autologous and allogeneic bone grafts have various limitations, which motivate the attempts to look for alternatives.

Synthetic biomaterials have undergone rapid development over recent decades, and now provide promising options for the repair of critical-sized bone defects. Under the requirement to fit the different shapes and mechanical microenvironments of bone defects, synthetic biomaterials have a wide variety of physicochemical properties, such as 3-dimensional geometry, chemical composition, and surface topography. However, hitherto, most of these biomaterials are neither intrinsically osteoinductive nor highly osteoconductive. This has greatly limited their clinical application in the repair of critical-sized bone defects. Even though many methodologies have been developed to improve these two properties of the biomaterials, their applicability is very limited **(Chapter 1)**.

Recently, we showed that our "BMP-2-incorporated biomimetic calcium phosphate (CaP) coating" was capable of rendering dental titanium implants both highly osteoconductive and efficaciously osteoinductive. In this thesis, we further showed that this technique could also be used to functionalize four kinds of polymers (Helistat®, Polyactive®, Ethisorb™ and PLGA) and deproteinized bovine bone (DBB, Bio-Oss® granules), whose physicochemical properties varied widely **(Chapter 2-6)**. These findings indicated a broad applicability of the technique in functionalizing biomaterials.

BMP-2-incorporated biomimetic CaP coating has two main merits. One is that the biomimetic coatings can incorporate a variety of bioactive agents through their co-precipitation. The coating-incorporated bioactive agents can be released slowly and sustainably *in vitro*. This release mode is of paramount importance to optimizing the osteoinductive efficacy of these agents. In an *in-vivo* environment, the incorporated protein can be released by three kinds of mechanisms: diffusion-controlled burst release, coating-chemical dissolution-controlled slow release, and multinucleated cells-mediated slow release **(Chapter 1)**. The other merit is that, when they are deposited on the abovementioned biomaterials, the biomimetic coatings have an identical geometric structure and chemical

composition. This property ensures that the coatings deposited on different biomaterials bear consistent biological characteristics **(Chapter 2)**.

For each polymer adopted in our study, the osteoinductive efficacy of BMP-2 was significantly higher when it was incorporated into the CaP coating than when it was directly adsorbed onto the surface of the material **(Chapter 3)**. We attributed the potentiated osteoinductive efficacy of BMP-2 that was incorporated into the CaP coating to its slow and sustained release. For the four polymers that were functionalized by this technique, the surface-area density of the polymers was the key factor in determining the bone regeneration.

Mechanical instability of bone defects can greatly compromise the process of bone regeneration. This principle fits to the bone regeneration associated with a directly adsorbed depot of BMP-2. After a 14-day subcutaneous implantation, the bone volume associated with the mechanically stabilized Ethisorb^{TM} (a flexible polymeric material) discs bearing an adsorbed depot of BMP-2 was ten times higher than that associated with their mechanically unstabilized counterparts. However, the bone volume induced by Ethisorb^{TM} discs bearing a coating-incorporated depot of BMP-2 was similar in the mechanically unstabilized and stabilized groups, and comparable to that induced by the stabilized Ethisorb^{TM} discs bearing an adsorbed depot of BMP-2. This finding indicated that, if an osteogenic agent is delivered in a cell-mediated manner (via coating degradation), ossification could be promoted even within a mechanically unstable environment **(Chapter 4)**.

The early osteogenic activities within Ethisorb^{TM} discs were also examined to determine the biological mechanism, whereby highly efficacious bone formation is induced by the coating-incorporated depot of BMP-2 **(Chapter 5)**. One of the most striking findings was of a characteristic ossification type – "BMP-2-incorporated coating-originated intramembraneous ossification" – within the Ethisorb^{TM} bearing a coating-incorporated depot of BMP-2. Such an ossification type was proved to be the main ossification type within Ethisorb^{TM} bearing a coating-incorporated depot of BMP-2. It contributed to the advantages of Ethisorb^{TM} discs bearing a coating-incorporated depot of BMP-2 in bone regeneration over the Ethisorb^{TM} discs bearing an adsorbed depot of BMP-2. In contrast, there was no significant difference in either the connective tissue-localized intramembraneous or the endochondral ossification among different groups.

Consistent with the data gleaned from the polymers, DBB bearing a coating-incorporated depot of BMP-2 could also induce bone formation significantly more efficiently than uncoated DBB bearing an adsorbed depot of BMP-2 **(Chapter 6)**. Another novel finding in the present thesis was that the interstitial space between the DBB bearing a coating-incorporated depot of BMP-2 and newly formed bone was filled with a significantly higher amount of bone marrow than the interstitial space of the uncoated DBB bearing an adsorbed depot of BMP-2. Since bone marrow is an important source of nutrients for osseous tissue and of pluripotent progenitor cells to sustain their turnover, its abundance bodes well for the health and endurance of the new bone.

The BMP-2-incorporated CaP coating was also consistently associated with a significant attenuation of the host inflammatory response. It could significantly reduce the volume densities of foreign-body giant cells and dense fibrotic capsule that were triggered by the implanted biomaterials. In contrast, the directly adsorbed depot of BMP-2 failed to do so.

The high effective dose of BMP-2 leads to a high cost of, and thus limits the clinical application of the BMP-2-incorporated CaP coating. BMP2/7 heterodimer has a significantly higher potency in inducing bone regeneration, and thus can be an ideal alternative to BMP-2. In our study, the optimal concentration of rhBMP2/7 heterodimer in inducing the migration and differentiation of preosteoblasts was significantly lower than those of the respective homodimers. After a 28-day *in-vitro* culture, the area of calcium depositions that were induced by 50ng/ml rhBMP2/7 was 12 or 38 times more than those were induced by 50ng/ml rhBMP2 or 50ng/ml rhBMP7 respectively. These findings confirmed the promising potential of BMP2/7 heterodimer for substituting BMP-2. This improvement will significantly reduce the cost of this technique and thus broaden its clinical application.

In conclusion, the “BMP-2-incorporated biomimetic calcium phosphate coating” is broadly applicable to functionalizing biomaterials. The coating-incorporated BMP-2 not only highly efficiently induces bone regeneration, but also suppresses the inflammatory reaction to the implanted biomaterials. The biomaterials that are functionalized using this technique can therefore be a promising treatment option in bone tissue engineering of critical-sized bone defects.